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INTERMEDIATE STATES IN THE PRECIPITATION OF HYDROXYAPATITE

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TO distinguish between the physico-chemical and physiological factors underlying the formation of biological hydroxyapatites, investigations have been undertaken in this laboratory on the ageing of aqueous preparation of synthetic calcium phosphates. In these studies, chemical and X-ray diffraction analyses are being employed to elucidate certain aspects of the nucleation and growth dynamics of the emerging solid phase under a wide variety of experimental conditions. The present article describes the precipitation behaviour of these calcium phosphates under conditions of high *p*H and high initial concentrations of reactants, and gives a description of some of the alterations which occur in the physical and chemical properties of the solid phase while in contact with the preparative solution.

To minimize the formation of more soluble acid phosphates, the following hydroxyapatite preparation, a modification of one proposed by Hayek and Stadlmann[1], was employed: 0·250 molar solutions of $(NH_4)_2HPO_4$ were rapidly added while stirring to 0·750 molar solutions of either $Ca(NO_3)_2.4H_2O$ or $CaCl_2.6H_2O$ to achieve a final phosphate concentration of 0·15 molar in the Ca/PO_4 molar ratio 1·71. All solutions were brought to a *p*H value of 10·5 with concentrated NH_4OH before mixing. The reactions were carried out at 25° C in closed systems to minimize CO_2 absorption. Constant stirring kept the slurry in intimate contact with the reaction solution for periods up to 2 weeks. Samples of the reaction slurry were withdrawn at fixed intervals after initial mixing for chemical, *p*H and X-ray diffraction analyses. To avoid changes in the solid during the drying of the slurry, all samples were lyophilized. The samples chosen for chemical studies were washed four times with ammoniated wash water (*p*H 10·5) before freeze-drying and then analysed for Ca by standard EDTA titration techniques and for PO_4 by the differential spectrophotometric technique of Gee and Deitz[2]. The supernatant and washings were also analysed for Ca and PO_4.

X-ray diffraction patterns of the samples were recorded on an electronic diffractometer equipped with a scintillation counter and a pulse-height analyser. Nickel-filtered copper $K\alpha$ radiation was employed. The scattering region

24–36°2θ was principally surveyed; this portion of the diffraction pattern includes the main hydroxyapatite diffraction profile. Because of extensive overlaps, only the 002 peak at 25·8°2θ was sufficiently resolved for line-breadth measurements. The width at one-half the maximum height of this peak, $\beta_{1/2}$, was measured in degrees 2θ and corrected for instrumental aberrations according to the method of Warren[3]. The quantity, $1/\beta_{1/2}$, which is proportional to the mean crystallite size and/or degree of perfection in the direction along the c-axis of the crystals was taken as the measure of the crystallinity index. Since it was observed qualitatively that variations in the degree of resolution of the remainder of the diffraction pattern parallel the changes in the 002 reflexion, this crystallinity index can be taken as representative of the crystal as a whole.

The initial solid phase, which separated immediately on mixing of the reactants, was 'non-crystalline' inasmuch as the diffraction patterns showed no discrete diffraction peaks (see Fig. 1*A*). The molar Ca/PO_4 ratio o this amorphous phase after washing was well below the 1·71 ratio of the overall reaction system and was found to be dependent on the Ca salt used in the preparation. The molar Ca/PO_4 ratio was remarkably consistent for any given salt; a ratio of 1·52 was found when the nitrate solution was used, a value of 1·46 was observed when the chloride solution was used. At most only 90 per cent of the total Ca available in solution was incorporated into the amorphous phase while the unincorporated Ca either remained in solution or was washed out of the precipitate. No detectable amount of PO_4 was found in either the reaction solution or the wash waters after solid formation.

X-ray diffraction patterns taken on the unwashed, freeze-dried product, as well as patterns taken directly of the slurry, centrifuged to remove excess water, established that the 'non-crystalline' character of the precipitate persisted in contact with the precipitating solution for several hours before completely transforming into a poorly crystalline hydroxyapatite (Fig. 1*B*). The observable conversion of the pre-apatitic phase into hydroxyapatite did not occur abruptly, but extended over a period of about 2 h. Measurements made on the 002 diffraction peak of samples collected during this period revealed that even though there was a continuous five-fold increase in relative peak area, indicative of a five-fold increase in converted apatite, the diffraction breadth remained unchanged at $1/\beta_{1/2} = 1{\cdot}44/°2\theta$ ($\sigma = \pm 0{\cdot}04$), a value smaller than normally found in poorly crystallized bone apatite[4]. When the pre-apatitic phase has fully converted, as evidenced by the constancy of the 002 diffraction peak area, a gradual sharpening of the 002 reflexion was observed ($1/\beta_{1/2} = 1{\cdot}75/°2\theta$ at 25 h, $1/\beta_{1/2} = 2{\cdot}39/°2\theta$ at two weeks).

Increase in the Ca/PO_4 molar ratio of the solid and

corresponding decreases in the concentration of solution Ca occurred concomitantly with the crystallographic conversion. By the end of two weeks the Ca/PO_4 molar ratio of the solid had increased to $1{\cdot}67 \pm 0{\cdot}01$, equalling that for pure hydroxyapatite and the Ca in solution had been considerably reduced from 10 per cent to about 2 per cent of the total Ca available in the solution at the start of the reaction. As before, no phosphate was detected in the reaction solution. An overall Ca/PO_4 molar ratio in slight excess of 1·67 was employed in these experiments to ensure that the final ratio of the solid was independent of the availability of solution Ca. The *p*H of the reaction solution, on the other hand, dropped from 10·5 to a value of 9·8 immediately on mixing and remained at this value during the entire period of crystallization. The crystal-chemical events described here were greatly accelerated when the reaction was conducted at 30·5° C instead of 25° C.

The ageing of freshly precipitated calcium phosphate as prepared under conditions described in this article can be divided into three stages (Fig. 2). The first stage, which commences immediately on mixing and continues for about 5 h, is characterized by an amorphous-like diffraction pattern which does not undergo any overt changes. Stage two is marked by the progression from a barely discernible apatitic profile superimposed on the amorphous pattern to a fully developed apatite pattern with the complete disappearance of any underlying amorphous features. The third stage covers the period following total conversion of the amorphous phase to a crystalline apatite and is characterized by the constancy of the 002

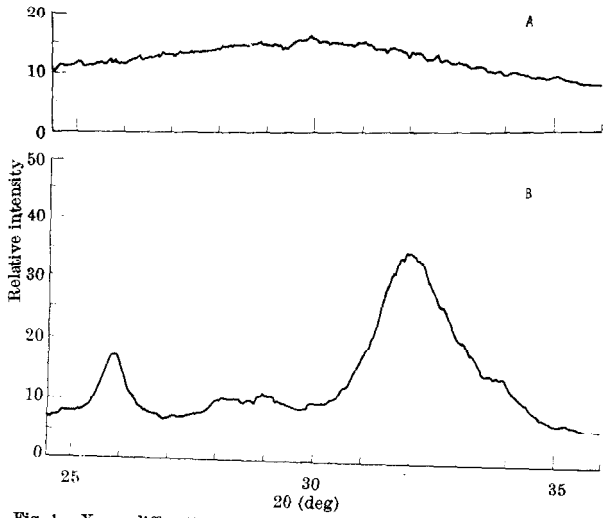

Fig. 1. X-ray diffraction patterns of (*A*) non-crystalline and (*B*) crystalline (apatitic) calcium phosphates (copper $K\alpha$ radiation)

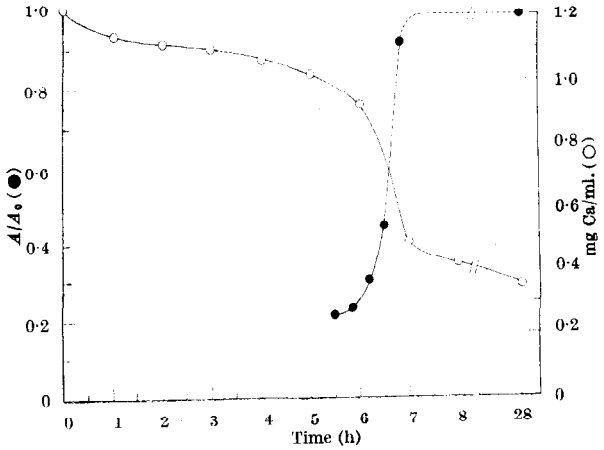

Fig. 2. The curve connecting the closed circles shows the per cent conversion of non-crystalline to crystalline calcium phosphate (A/A_s) as a function of time. The plot connecting the open circles indicates the drop in solution Ca concentration (mg Ca/ml.) with time

peak area. This final stage commences about 7 h after the reaction is initiated, and continues indefinitely.

Despite the apparent constancy in the diffraction pattern, the first stage is not chemically static. Slight but significant decreases in solution Ca occur during this period (from 1·20 mg/ml. to 1·02 mg/ml.; Fig. 2). Moreover, the demarcation between stages one and two is arbitrarily based on the initial appearance of a discrete apatite diffraction pattern. Since the 002 peak area at the beginning of the second stage is already about 1/5 the value achieved at maximum conversion, it is clear that about 20 per cent of the amorphous material has been converted to the crystalline phase by the time stage two can be detected. Thus, crystallographic as well as chemical changes are taking place in this initial period. Nearly coincident with the emergence of a discernible diffraction profile, however, is a more rapid decrease in solution Ca-levels (from 1·02 mg/ml. at the beginning of stage two to 0·42 mg/ml. at the end; Fig. 2). In addition, the 002 diffraction peak, once discernible, proceeds to develop at a rate commensurate with the accelerated rate of fall in solution Ca-levels, so that the remaining 80 per cent of pre-apatite converts over a much shorter interval than the initial 20 per cent. One can conclude, then, that while no fundamental distinction may exist between stages one and two, a distinction between rates of conversion can be made with validity, with the crystal phase formation taking place at a much accelerated pace in the second stage than in the first. The constancy of the 002 crystallinity values up to the end of the second stage suggests that the rate of conversion of the amorphous phase in stage two depends

on the nucleation of new crystals rather than the growth or perfection of pre-existing crystals.

The most characteristic feature of the third stage is the gradual increase in crystallinity. The improvement in hydroxyapatite crystallinity during this period is typical of the gradual enlargement of mean particle size of many low-solubility precipitates as a result of re-precipitation in solution. This growth of the precipitate, known as Ostwald ripening, is generally attributed to the dissolving of the smaller, more soluble crystals and re-deposition of the dissolved material on the growing faces of the larger, less-soluble crystals[5]. No doubt this re-crystallization is in progress from the earliest stages of the conversion, but the time interval in stage two is too short for this gradual increase in crystal dimensions to have a measurable effect on the crystallinity values measured during this period. The gradual decrease in solution Ca from 0·42 to 0·20 mg/ml. during this final stage indicates that a perfection of the crystalline phase parallels the crystal growth.

To describe the initial phase as 'non-crystalline' is to imply only that there has been insufficient development of a regular, repetitive, spatial array of ions to define a crystalline structure. This does not preclude the possibility of localized order and structure. Indeed, the fact that the molar Ca/PO_4 ratio does not vary under a given set of initial conditions suggests a chemically well-defined local structural unit.

The formation of 'non-crystalline' calcium phosphate is a kinetic phenomenon. The rapid mixing of the reaction solutions creates strong interactions between Ca^{++} and $HPO_4^{=}$ ions leading to irregular co-ordination complexes large enough in size to separate from solution. The subsequent temperature-dependent transformation into apatite indicates that this initial phase is isothermally metastable with respect to the more ordered apatite configuration. In addition, the sigmoid-shaped relationship of the transformation with time strongly suggests that the conversion mechanism is autocatalytic. It is possible that the interfacial surfaces of the emerging crystalline phase act as sites for heterogeneous nucleation, thereby accelerating the rate of conversion. In this regard, the 'non-crystalline' calcium phosphate remains stable indefinitely if kept dry, pointing out the necessity for water for the process of nucleation to take place in this system.

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